Enzyme Immunoassay for the Rapid Detection of *Escherichia coli* O157

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Abstract: An enzyme immunoassay(EIA) to detect *Escherichia(E.) coli* O157 in pork was developed by using a sandwich-type assay on the 96-well microplates. All *E. coli* O157 strains and *Citrobacter amalonaticus* reacted strongly, however 29 *E. coli* serotypes and 15 non-*E. coli* bacterial strains showed negative in *E. coli* O157 EIA. *E. coli* O157 in pork could be detected within 13 h including 10 h in enrichment broth and 3 h in EIA. As few as 1.8 CFU of *E. coli* O157 per g of pork could be detected after enrichment, whereas above 1.8×10^5 CFU of *E. coli* O157 per g of pork could be detected without enrichment. The *E. coli* O157 EIA was a sensitive, easy-to-perform and efficient method for the screening of *E. coli* O157 in pork.

Keywords: EIA, *E. coli* O157, screening

Introduction: Since *E. coli* O157:H7 was first recognized as a pathogen in 1982, it has become one of the most significant food-borne pathogens. It was necessitated to develop the screening methods that could detect the low numbers of *E. coli* O157:H7 in food. The lack of sensitivity of direct plating procedures has led to develop of enrichment broth that could multiply *E. coli* O157:H7 to detectable levels before rapid detection or culture isolation. Because of simplicity, speed and high-volume testing, EIA was widely used for screening of food-borne pathogens. The purpose of this study was to develop a rapid, easy-to-use, sensitive and specific EIA to detect *E. coli* O157 in pork.

Materials and Methods: EIA was performed in 96-well Nunc-Maxisorp™ plates. Anti-rabbit *E. coli* O157 polyclonal antibodies were produced by the Ewing's methods(1986) and purified with immunoaffinity chromatography. The purified antibodies were coated at a concentration of 0.5 ug/well and the remaining binding sites were blocked with 2 % skim milk. One ml of enriched sample was

mixed with 50 ul of 10 % Triton X-100 and then heat-treated at 100 . for 20 min in a water bath. Heat-treated samples were placed into EIA wells and incubated for 2 h. After washing with PBST, 10 ng of peroxidase labeled *E. coli* O157 antibodies was added and incubated for 30 min followed by a second washing and the addition of TMB substrate. An OD650 of 0.3 above background was considered as positive. Specificity of EIA was evaluated with pure-cultured 48 bacterial strains and sensitivity was determined with pork that was inoculated with *E. coli* O157:H7 at concentration ranging from 1.8 $\times 10^7$ CFU/g to 1.8 CFU/g. During the enrichment, aliquots of cultures were tested at every 5 h-interval to determine the minimum-enrichment time for *E. coli* O157 EIA. Eighty-five pork samples were attempted to detect of *E. coli* O157 by using EIA as well as to isolate of *E. coli* O157:H7 by culture methods, simultaneously.

Results: Regardless of H serotype, *E. coli* O157 strains(*E. coli* O157:H7; H19; nonmotile) reacted strongly in the EIA, whereas the others representing 29 *E. coli* serotypes were all negative. Among 16 non-*E. coli* bacterial strains, only *Citrobacter amalonaticus* was EIA-positive. Samples inoculated with 1.8 *E. coli* O157:H7 per g of pork showed EIA-positive after 10 h enrichment(Table 1). Two (2.4 %) of the 85 pork samples were positive in EIA but *E. coli* O157:H7 was not isolated from EIA-positive as well as EIA-negative samples.

E. coli O157:H7 (CFU/g)*	Range of OD650 (score) of enrichment culture-EIA in indicated incubation time (h)			
	0	5	10	15
1.8 $\times 10^7$	0.79 . 1.25 (.)	0.86 . 1.04 (.)	0.80 . 1.06 (.)	1.19 . 1.39 (.)
1.8 $\times 10^6$	0.53 . 0.68 (.)	0.86 . 0.98 (.)	0.76 . 1.04 (.)	1.24 . 1.34 (.)
1.8 $\times 10^5$	0.30 . 0.52 (.)	0.73 . 0.75 (.)	0.74 . 1.06 (.)	1.27 . 1.37 (.)
1.8 $\times 10^4$	0.12 . 0.24 (.)	0.44 . 0.46 (.)	0.80 . 1.12 (.)	1.31 . 1.36 (.)
1.8 $\times 10^3$	0.07 . 0.09 (.)	0.23 . 0.25 (.)	0.78 . 1.11 (.)	0.87 . 1.15 (.)
1.8 $\times 10^2$	0.07 . 0.08 (.)	0.13 . 0.15 (.)	0.79 . 1.04 (.)	0.64 . 0.86 (.)
1.8 $\times 10^1$	0.07 . 0.08 (.)	0.08 . 0.09 (.)	0.66 . 0.67 (.)	0.63 . 0.79 (.)
1.8 $\times 10^0$	0.03 . 0.06 (.)	0.08 . 0.09 (.)	0.44 . 0.56 (.)	0.66 . 0.83 (.)
Negative control**	0.03 . 0.04 (.)	0.08 . 0.09 (.)	0.09 . 0.10 (.)	0.07 . 0.08 (.)

* No. of *E. coli* O157:H7 inoculated in pork

** Negative control was used with enriched-pork that was not inoculated *E. coli* O157:H7

Table 1. Results of enrichment culture-EIA for detection of *E. coli* O157 in pork

Discussion: EIA had various advantages including high sensitivity, easy-to-use and rapid detection, whereas the major disadvantage was its limited specificity for the use of polyclonal antibodies.

It was reported that some of the non-*E. coli* strains, for example *Citrobacter freundii* and *E. hermanni*, shared common antigens with *E. coli* O157 (Bettleheim et al., 1993; Borczyk et al., 1990). However, the disadvantage could be overcome by following selective plating media; *Citrobacter amalonaticus* was inhibited the growth on CT-SMAC as well as fermented sorbitol.

Minimun number of *E. coli* O157 detectable in EIA was 1.8 CFU/g with 10 h enrichment and 1.8×10^5 CFU/g of pork without enrichment. Accordingly, it was necessiated for enrichment procedure to detect small numbers of *E. coli* O157. EIA-positive samples that were not isolated *E. coli* O157:H7 might be contained the bacteria reacted with *E. coli* O157 antibodies.

E. coli O157:H7 was not isolated from EIA-negative samples and it meaned that there were no false negative reaction in this EIA. Consequently, *E. coli* O157 EIA-negative samples were not required for culture confirmation of *E. coli* O157:H7, whereas only EIA-positive samples were considered to be presumptive until confirmed by culture.

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